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Article

Effect of relative humidity on the efficacy of entomopathogen *Beauveria bassiana*-based mycopesticide against red spider mite *Tetranychus macfarlanei*

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Abstract

Tetranychus macfarlanei is a potential threat to various economically important crops. Chemical control is widely used to control this spider mite, but the exclusive reliance on chemical acaricides is now being questioned, and entomopathogens are emerging as a promising alternative. The efficacy of the entomopathogens largely depends on abiotic factors like temperature, humidity, rainfall etc. The experiments were conducted to determine the effect of relative humidity on the efficacy of the entomopathogen *Beauveria bassiana* (strain GHA), marketed as BotaniGard ES, against the adult female and egg stages of *T. macfarlanei* under laboratory conditions. Adult females and eggs of *T. macfarlanei* were treated with three concentrations of BotaniGard (1×10^6, 1×10^7, and 1×10^8 conidia/ml), and virulence was assessed on mites held at three relative humidity levels (55, 75, and 95 ± 2% RH) at 25 ± 1 °C. The results showed that the mortalities of adult females and eggs of *T. macfarlanei* were dose-dependent, and there is certainly a synergistic effect of relative humidity on the efficacy. When the eggs were treated under the above laboratory conditions, highest egg mortalities of 86.4 and 91% were recorded at a concentration of 1×10^8 conidia/mL at 75 and 95 ± 2% RH, respectively, after six days of treatment. The LT_{50} values on adult females were 28.7 h and 33.4 h at 95 and 75 ± 2% RH in a concentration of 1×10^8 conidia/ml, respectively, which was significantly lower than values for other concentrations and RH levels. This study identified 1×10^8 conidia/ml of *B. bassiana* at 75 to 95 ± 2% RH as the best possible combination than other lower RH to manage the spider mite.

Keywords: Tetranychidae, Entomopathogen, Abiotic Factors, Biological Control, Synergy

Introduction

Tetranychus macfarlanei Baker and Pritchard is a cosmopolitan pest. It is distributed in Afrotropical, Oriental, and Palearctic regions in different countries such as Bangladesh, the Canary Islands, China, India, Malaysia, Madagascar, Mauritius, the Phillippines, and Thailand (Ullah *et al*. 2021; Migeon & Dorkeld 2022). Because of its' phytophagous nature, it infests a wide range of economically

important crops belonging to the families Malvaceae, Fabaceae, Cucurbitaceae, Convolvulaceae, and Solanaceae (Jeppson *et al*. 1975; Bolland *et al*. 1998). *Tetranychus macfarlanei* has arrived lately in Bangladesh, but in this short period, it has spread throughout the country, and is threatening many economically important crops, including jute, bean, eggplant, and bottle gourd (Ullah *et al*. 2012, 2021).

In Bangladesh, farmers have widely used insecticides and acaricides to control spider mites (Ullah & Gotoh 2013). However, chemical control seems to lose its' effectiveness, probably due to spider mites which may rapidly develop resistance to various acaricides after only a few applications (Whalon *et al*. 2021). Several unique biological characteristics of spider mites such as rapid population growth, high fecundity, and arrhenotokous reproduction contribute to their rapid development of acaricide resistance (Van Leeuwen *et al*. 2009). The Arthropod Pesticide Resistance Database (APRD) reports nearly 977 cases of acaricide resistance in phytophagous mites from the family Tetranychidae (Mota-Sanchez & Wise 2022). Therefore, sole dependence on insecticides is not a sustainable option for controlling tetranychids. To overcome the resistance problem, farmers increase spraying. Consequently, problems like high spraying costs, environmental pollution, and adverse effects on beneficial organisms may occur. The application of microbial pathogens, especially entomopathogenic fungi, is a promising alternative for successful pest management. Moreover, the mites do not develop resistance against fungi. They also have no toxic effects on environment, and have the potential for future biotechnological developments (Al Khoury *et al*. 2019, 2020).

Fungal entomopathogens have been performing well for several decades to get established as an important component in pest management programs worldwide (Lacey *et al*. 2015). About 700 species in 90 genera have been considered insect-pathogenic fungi (Khachatourians & Qazi 2008). Some of the most commonly studied fungal species are *Beauveria bassiana* (Balsamo) Vuillemin, *Cordyceps fumosorosea* (Wize) Kepler, B. Shrestha & Spatafora (previously known as *Isaria fumosorosea* Wize), *Metarhizium anisopliae* (Metschnikoff) Sorokin, and *Lecanicillium lecanii* (Zimmerman) Viegas (Li *et al*. 2011; Chen *et al*. 2015).

Beauveria bassiana has gained much popularity as a biological control agent for many insect pests, including ticks and mites, throughout the world (Chandler *et al*. 2000). However, its potential as a mycoacaricide has caught attention recently (Tamai *et al*. 1999, 2002). It has been an effective pathogen against many arthropod pests, including spider mites (Faria & Wraight 2001; Feng *et al*. 2004; Hatting *et al*. 2004; Pu *et al*. 2005; Ullah & Lim 2015). The repeated application of this fungal entomopathogen causes detrimental effects on certain arthropod pests and, thus, keeps the pest population under control (Groden *et al*. 2002). However, different strains of *B. bassiana* were found effective against spider mites (Chandler *et al*. 2005). The efficacy of *B. bassiana* BotaniGard strain against *T. macfarlanei* is reported as promising and suitable for managing this pest (Tahmina *et al*. 2020). For the appropriate management of spider mites, the interest in mycoacaricides has increased in recent years, especially *B. bassiana* is proven trustworthy (Alves *et al*. 2002, 2005; Shi & Feng 2006, 2009; Seiedy *et al*. 2010). However, the effectiveness of entomopathogenic fungi is regulated by several abiotic factors such as temperature, humidity, and solar radiation (Benz 1987; Inglis *et al*. 2001; Alves *et al*. 2005; Seiedy *et al*. 2010), host population level, and the presence of antagonists (James *et al*. 2003; Toledo *et al*. 2011). Some studies have shown that the temperature, humidity, UV radiation, etc., separately causes a reduction in the viability and virulence of the conidia after application, resulting in poor performance by the entomopathogenic fungi (Zimmermann 2007). Successful use of mycoinsecticides relies particularly on ambient relative humidity (RH) conditions and the concentration of conidia applied (Ferron *et al*. 1991; Alves *et al*. 2005; Devi & Rao 2006). The development of an efficient management strategy for controlling spider mites by using a particular *B. bassiana* strain requires a proper combination of both the conidial concentration and

relative humidity conditions. Therefore, this study was designed to determine the effect of relative humidity on the efficacy of *B. bassiana* against *T. macfarlanei*.

Materials and methods

Collection and rearing of Tetranychus macfarlanei

The mite strain of *T. macfarlanei* was collected from a brinjal plant (*Solanum melongena* L.) (Solanaceae) in Dhaka, Bangladesh (23°82′N, 90°38′E) on 27 March 2020. They were maintained on bean (*Dolichos lablab* L.) leaves (ca. 25 cm^2) kept in contact with water-saturated polyurethane mats in plastic Petri dishes (90-mm diameter, 20-mm depth) at 25 ± 1 °C, 65 ± 5% relative humidity, and 16L: 8D h photoperiod. The edges of the bean leaves were covered with moist tissue paper through watering and prevented mites from escaping. The leaves were replaced when they were exploited by the feeding of spider mites (Ullah *et al*. 2014).

Fungal pathogen and preparation of conidial suspension

A commercially available entomopathogenic fungus, *B. bassiana*, strain GHA contained 1.6×10^{10} fungal conidia/ml (BotaniGard® ES; Arysta LifeScience, Tokyo, Japan) was used in the bioassay tests. Suspension was prepared by diluting of formulated mycopesticide BotaniGard containing strain GHA of *B. bassiana* at concentration 1.6×10^{10} conidia/ml. Conidial suspensions were vortexed for 5 min, and spore concentrations were determined using a haemocytometer (Neubauer-improved haemocytometer, Lauda-Königshofen, Germany). The viability of conidia was determined before the bioassay by spread-plating 0.1 ml of conidial suspension titrated to 1×10^4 conidia ml^{-1} on SDA plates. Plates were incubated at 25 ± 1 °C, and the percentage germination was determined after 24 h from 100-spore counts by placing a sterile microscope coverslip on each plate under a stereomicroscope (SZ40, Olympus Corporation, Tokyo, Japan) (Ullah & Lim 2017). Each plate was replicated 4 times. Conidia germination > 90% was observed in all tests. The commercial bottle was shaken properly and the conidial suspensions, 1×10^8 conidia/ml, 1×10^7 conidia/ml, and 1×10^6 conidia/ml were prepared using distilled water.

Achieving different relative humidity (RH) regimes

Three different RH regimes, 55, 75, and 95 ± 2% achieved by dissolving $Mg(NO_3)_26H_2O$, NaCl, and K_2SO_4 in distilled water were used in the experiments, respectively (Rockland 1960). Salt solutions were dissolved in water in a beaker, and the mixture was placed on a magnetic heating stirrer. The solution was allowed to cool and then poured down into desiccators. The RH was measured using a data logger (Thermometer and Hygrometer, SKU KUT527, Daiso, Japan).

Effects of Beauveria bassiana *BotaniGard strain against* T. macfarlanei *eggs under different humidity*

The three to 5-day-old gravid females of *T. macfarlanei* were transferred on bean leaf discs (ca. 16 cm^2) placed with their lower side up in contact with water-saturated polyurethane mats in plastic Petri dishes (9 cm diameter). The spider mites were allowed to lay eggs for 24 h at 25°C, 60–70% RH, and a 16:8 h L: D photoperiod. Then the females were removed, and eggs were counted on each leaf disc. Subsequently, the test units were sprayed with the three concentrations (1×10^6, 1×10^7, and 1×10^8 conidia/ml) of *B. bassiana* (1 ml/cm^2) by using a hand sprayer (52G-20CM-IMP, RFL best buy, Bangladesh). After air drying, the leaf discs with eggs were kept under three RH regimes (55, 75, and 95 ± 2%), all at 25 ± 1°C, and a 16:8 h L: D photoperiod. Mortality was recorded 144 h following the application, and eggs that did not hatch were scored as "dead". The duration of the egg

stage until larval hatching is less than 130 h for *T. macfarlanei* (Ullah *et al*. 2012). A total of 342–374 eggs obtained from 20 females were used for each humidity. A group of test units sprayed using distilled water served as the control.

Effects of Beauveria bassiana *BotaniGard strain against* T. macfarlanei *adult females under different humidity*

Three to 5-day-old gravid females of *T. macfarlanei* were transferred on bean leaf disc (ca. 16 cm^2) placed in plastic Petri dishes as described earlier. The test units were incubated for 24 h. Dead or injured individuals were then removed, and the test units were sprayed with the three concentrations described above. After air drying, the mite-infested discs were kept under three RH regimes (55, 75, and 95 ± 2%), all at 25±1°C and a 16:8 h L: D photoperiod in an incubator until the mites' death. Mites that did not move their appendages when touched with a fine brush were considered dead, and mortality was recorded. Four replicates, each with 20 individuals for each fungal concentration and humidity regime, were used in the experiments. A group of test units sprayed using distilled water served as the control.

Data analysis

The percentages of dead eggs and adult females were corrected using the formula suggested by Abbott (1925):

Corrected mortality (%) = [100×(Treated − Control) / (100 − Control)]

The LT_{50} and LT_{90} were determined by Probit analysis using POLO-Plus (LeOra software). The correction of overlapping confidence intervals of the LT_{50} was used to establish whether lines were significantly different at the 5% level (Robertson *et al*. 2007). Before analysis, the values (egg) were arcsine transformed to normalize the data. A test for significant variation in the mean egg mortality was performed to reveal interactions between humidity and concentration factors by two-way analysis of variance (IBM 2019).

Results

Effect of relative humidity on the efficacy of Beauveria bassiana *against eggs of* Tetranychus macfarlanei

Humidity and concentrations of *B. bassiana* significantly affect the mortality of *T. macfarlanei* eggs (humidity F=23.85, df=2, 27, $P < 0.001$; concentration F=31.10, df=2, 27, $P < 0.001$), but their interaction was not significant (humidity×concentration F=1.57, df=2, 27, P=0.210). The egg mortality was significantly higher at 95 and 75% RHs than at 55% RH at the same concentration (Fig. 1). The mortality in control was ≤ 5% in all concentrations. The corrected egg mortality was 91, 86.4, and 72% at 95, 75, and 55% RHs, respectively, with the same conidial concentration of 1×10^8 conidia/ml. The egg mortality increased with the increasing conidial concentration from 1×10^6 to 1×10^8 conidia/ml at the same RH condition. Mortality greatly increased with increasing RH only at the highest conidial concentration of *B. bassiana*.

Effect of relative humidity on the efficacy of Beauveria bassiana *against adult females of* Tetranychus macfarlanei

Adult mortality greatly increased with increasing RH and at a higher conidial concentration of *B. bassiana*. At 95% RH, the LT_{50} values were 28.7, 48.6, and 76.1 h at 1×10^8, 1×10^7, and 1×10^6 conidia/mL, respectively, which was not significantly different at 75% RH when compared with the same conidial concentrations of *B. bassiana* (33.4, 51.9, and 79.9 h, respectively; Table 1). At 55%

RH, the LT_{50} values were 52.6, 91.4, and 130.1 h at the respective conidia/ml, which was significantly higher than other RHs despite the individual concentrations. The LT_{90} values at different RHs also showed a similar trend to LT_{50}.

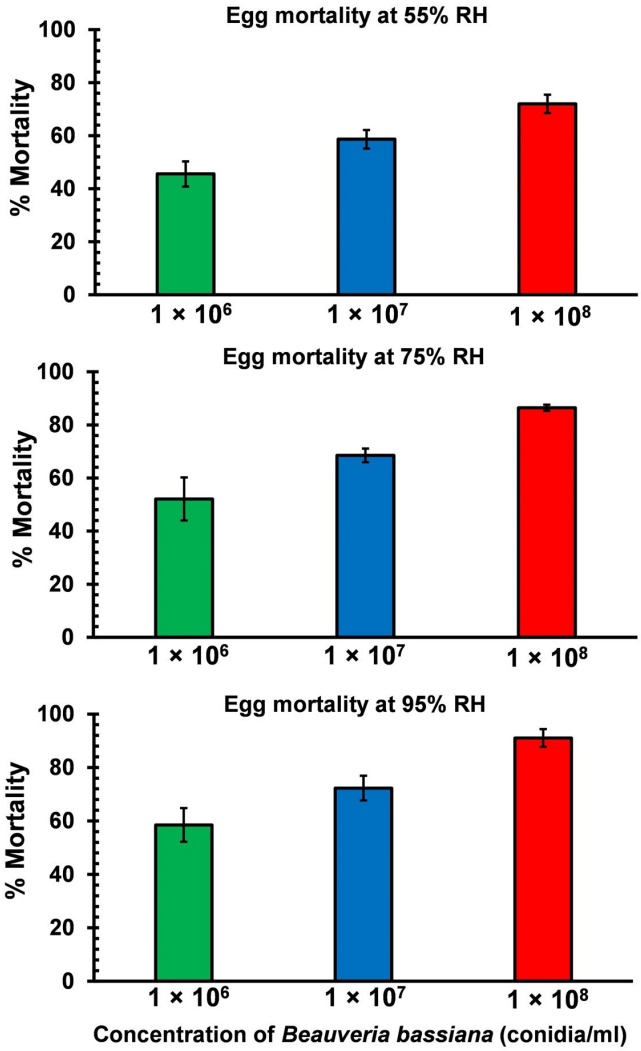

FIGURE 1. Corrected egg mortality of *Tetranychus macfarlanei* exposed to different concentrations of *Beauveria bassiana* at 95, 75 and 55% relative humidity conditions at 25 ± 1ºC. Error bars indicate 95% confidence intervals.

Relative humidity at different conidial concentrations has a significant impact on the survivorship of adult females. The survivorship of adult females was decreased with increasing RH along with higher conidial concentrations. The survival was higher in lower conidial concentrations recorded at 17 days, 12 days and 8 days at 1×10^6, 1×10^7, and 1×10^8 conidia/ml, respectively, with the same RH of 95 ± 2% (Fig. 2). The LT_{50} of adult female survival was also decreased as conidial concentrations increased at 75% RH (79.4, 51.9 and 33.4 h at 1×10^6, 1×10^7, and 1×10^8 conidia/ml, respectively) and 55% RH (130.1, 91.4, and 52.6 h at 1×10^6, 1×10^7, and 1×10^8 conidia/ml, respectively).

TABLE 1. Susceptibility of adult females of *Tetranychus macfarlanei* to *Beauveria bassiana* at different relative humidity levels.

RH	Concentrations	N[a]	LT_{50} (95% CL)[b]		LT_{90} (95% CL)[b]		Slope ± SE	χ^2	df
95% RH	1×10^8	80	28.70a	(22.53–34.24)	71.69a	(58.50–97.86)	3.22 ± 0.24	24.74	6
	1×10^7	80	48.61b	(41.10–56.01)	127.96b	(105.19 –171.10)	3.05 ± 0.20	38.58	9
	1×10^6	80	76.13c	(65.64–86.81)	199.30bc	(164.56–263.48)	3.06 ± 0.16	82.99	14
75% RH	1×10^8	80	33.41a	(28.57–37.99)	94.44ab	(80.96 –115.72)	2.84 ± 0.19	17.94	8
	1×10^7	80	51.95b	(46.80–56.94)	122.68b	(109.34–141.69)	3.43 ± 0.19	25.55	11
	1×10^6	80	79.94c	(72.17–87.82)	237.10cd	(203.64–289.04)	2.71 ± 0.15	39.92	15
55% RH	1×10^8	80	52.59b	(44.93–60.18)	132.99b	(110.87–172.81)	3.18 ± 0.19	45.42	10
	1×10^7	80	91.38c	(81.62–101.45)	251.22cd	(212.12–316.81)	2.92 ± 0.15	64.62	16
	1×10^6	80	130.14d	(104.71–160.40)	333.18d	(246.85–597.71)	3.14 ± 0.15	410.77	20

Note: LT_{50}, 50% lethal time; CL, confidence limits;
[a] Total number of mites used;
[b] Lethal time (95% confidence limits).
The correction of overlapping confidence intervals of the LT_{50} and LT_{90} was used to establish whether lines were significantly different at the 5% level (Robertson *et al*. 2007).
SE=Standard Error bars indicate 95% confidence intervals.

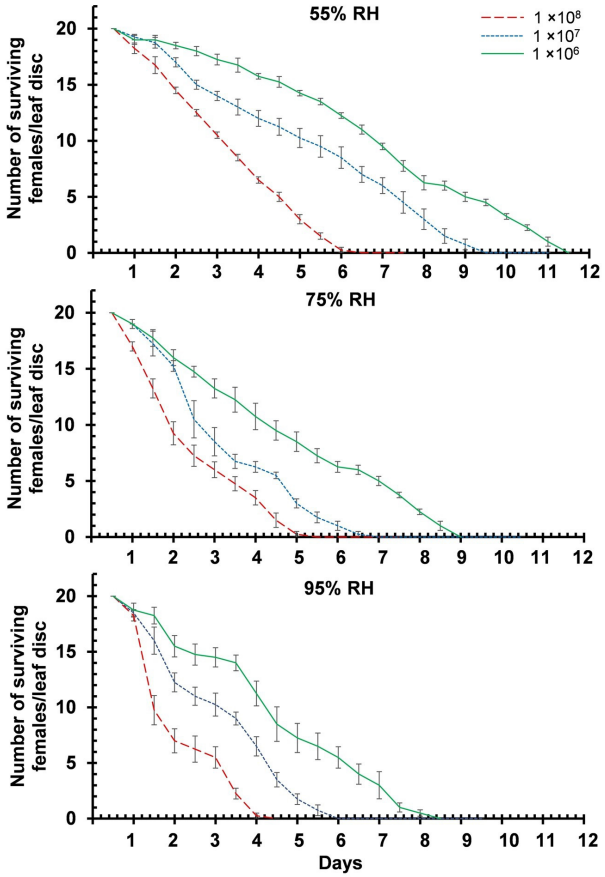

FIGURE 2. Survivorship of adult females of *Tetranychus macfarlanei* exposed to different concentrations of *Beauveria bassiana* at 95, 75, and 55% relative humidity conditions at 25 ± 1ºC.

Discussion

The experiments were conducted to evaluate the effect of relative humidity on the efficacy of the fungal pathogen *B. bassiana* against the spider mite, *T. macfarlanei*. The effect was observed on the egg and adult mortalities of the spider mite by creating different humidity conditions under laboratory conditions. The results revealed that humidity had a positive effect on efficacy of mycopesticide. The best result was obtained at a concentration of 1×10^8 conidia/ml under 75 to 95% relative humidity conditions.

Several studies have also revealed the effect of *B. bassiana* against spider mites, including *T. macfarlanei*, at different concentrations. The response of adult female *T. macfarlanei* was dependent on the concentration of *B. bassiana*; the LC_{50} and LC_{90} values were 3.63×10^7 and 2.68×10^8 conidia/ml, respectively (Tahmina *et al*. 2020). *B. bassiana* was reported as highly effective against the eggs of *Tetranychus urticae* Koch under laboratory conditions (Basak *et al*. 2021). Beauvericin, a *B. bassiana* mycotoxin, was reported to cause 100% mortality on motile stages of *T. urticae* at a concentration of both 100 and 1000µg/g, while inhibited egg hatching up to 69.3 and 83.3%, respectively (Al Khoury *et al*. 2019). *B. bassiana* MK918495 isolate caused 27.6 to 89.2% mortality in *T. urticae* 7 days after treatment at varying concentrations ranging from 4×10^4 to 4×10^9 conidia/ml (Athisintha *et al*. 2019). *B. bassiana* isolate B2 was reported to be effective against *T. urticae* as it caused 70, 80, and 84% mortality at 10^5, 10^6 and 10^7 conidia/ml concentrations, respectively (Ahmad *et al*. 2018). Among 12 isolates of *B. bassiana* applied in a study against *T. urticae*, SCWJ2, SDDZ-9, LNSZ-26, GZGY-1-3, and WLMQ-32 strains were reported as the most potent; they caused 37.6–49.5% corrected adult mortality at a concentration of 1×10^7 conidia/ml after 4 days following the treatment (Wu *et al*. 2016). The susceptibility of different developmental stages of *T. urticae* to the entomopathogenic fungi *B. bassiana* was evaluated under laboratory conditions. The study revealed that the highest conidial concentration (1×10^7 conidia/ml) significantly reduced the viability of eggs and increased the mortality of motile stages (Bugeme *et al*. 2014). *B. bassiana* Naturalis-L reduced around 97% of *T. urticae* population in a tomato greenhouse when applied at a rate of 1×10^8 conidia/ml (Chandler *et al*. 2005). It has been reported that *B. bassiana* has the potential to suppress future generations of *Tetranychus ludeni* Zacher by reducing its oviposition rate (Pereira *et al*. 2019).

The effectiveness of *B. bassiana* against spider mites in some studies showed poor performance. Some isolates of *B. bassiana* having 1×10^7 conidia/ml concentration showed poor performance against the eggs, immatures, and adults of *T. urticae* where laboratory bioassays showed their corrected mortalities were 2.7–3.8, 17.5–25.8, and 63.2–71.2%, respectively (Wu *et al.* 2019). A relatively higher concentration of 1×10^8 conidia/ml, *B. bassiana* 432.99 and Naturalis-L isolates caused lower rates of mortality of *T. urticae* under laboratory conditions, and the mortality recorded were 46.2–72.2% and 52.1–95.2%, respectively (Chandler *et al*. 2005). *B. bassiana* isolate 447 was found ineffective against *T. urticae* in a laboratory assay. Even at a higher concentration of 1×10^9 conidia/ml and 70±5% RH, only 51.7% mortality occurred (Andreleva & Shternshis 1995; Tamai *et al*. 1999). Other isolates of *B. bassiana* showed poor performance against *T. urticae*; when used at the rate of 1×10^7 conidia/ml, it causes 16–33% mortality following six days after inoculation (Chandler *et al.* 2005). A series of factors such as experimental condition, host type, application interval, temperature, humidity, rainfall, etc., plays a vital role in the virulence of the *B. bassiana*, and they may be responsible for this variation in the efficiency of the entomopathogen. The DNA characteristics and enzymatic activity of *B. bassiana* also significantly impact the outcome (Almeida *et al*. 1997; Moino *et al*. 1998).

In the case of spider mites, the effect of relative humidity on the efficacy of *B. bassiana* was reported in some experiments. A laboratory bioassay of *B. bassiana* against *T. urticae* on leaf discs

and potted bean plants revealed that the LT_{50} value was significantly lower at 1×10^8 conidia/ml and 95% RH than values for other lower RH levels at the same conidial concentration. A 1×10^7 conidia/ml concentration followed a similar trend (Ullah & Lim 2015). In the greenhouse, *B. bassiana* causes a higher percentage of infection (60–88.8%) to mites and insects under 97.5% RH, while at 75 and 80% RH only 15.3–43.9% of them were infected. A fifteen percent increase in RH results into 17–25% increase in the percentage of infection (Shipp *et al*. 2003). The 35% increase in the humidity level caused 8–12% increase in the mortality for *B. bassiana* AT076 on the 3rd and 5th days after application, respectively (Ortucu & Algur 2017).

Our results showed that infection of *T. macfarlanei* by *B. bassiana* was highly dependent on the conidial concentrations and the RH conditions. But a conidial concentration lower than 1×10^8 conidia/ml caused lower mortality irrespective of RH values. The test mites are small, and their cryptic living works (silky thread) are a barrier to efficient use of the applied concentration. As a result, there might be less possibility of contact in concentration below the threshold. The mites' defense mechanism triggers phagocytosis, melanization, or encapsulation responses as soon as harmful foreign molecule enters. So, if a lower number of conidia encounters the insect cuticle, it gets easier for the mites' immune system to combat them. No infection is thus apparent when the conidial dose is lower than the threshold (Devi & Rao 2006). It has been reported that a certain amount of pathogen concentration is required for successful infection, such as 1×10^7 conidia/ml *B. bassiana* against *Metarhizium anisopliae* (Metschnikoff) Sorokin (Coleoptera: Meloidae) (Devi & Rao 2006), 1.6×10^8 conidia/m^2 of *M. anisopliae* (Ascomycota: Hypocreales) against *Anopheles gambiae* Giles and *Culex quinquefasciatus* Say (Diptera: Culicidae) (Scholte *et al*. 2003), and 1×10^6 conidia/ml of *M. anisopliae* isolate Qu-M984 against *Pseudococcus viburni* (Signoret) (Hemiptera: Pseudococcidae) (Pereira *et al*. 2011). Moreover, higher concentrations of *B. bassiana* are known to infect *Lygus hesperus* Knight (Hemiptera: Miridae) even at lower humidity (Dunn & Mechalas 1963). Viable fungal conidium is a prerequisite for germination and infection. Germination percentage in vitro determines fungal virulence (Altre *et al*. 1999). The conidial germination largely depends on RH and temperature (Feng *et al*. 1994; Roberts & St. Leger 2004), and spider mite eggs are usually laid on the surfaces of leaves with some moisture (e.g., metabolic water, dew). The high egg mortalities caused by *B. bassiana* at the same regimes may be interpreted as the effect of egg moisture or humidity that facilitated germination and increased the virulence of the entomopathogen *B. bassiana*. This indicates that the emulsifiable formulation would be an effective choice for the use of *B. bassiana* against spider mites under field conditions. The results of the current study indicated that infection of *T. macfarlanei* eggs and adults caused by *B. bassiana* was highly dependent on relative humidity and conidial concentration. The leaf disc assay showed that a concentration of *B. bassiana* of 1×10^8 and a RH of 95 ± 2% was very effective for managing *T. macfarlanei*. The LT_{50} and LT_{90} values were 28.7 h and 71.69 h, respectively. This period was significantly shorter than those for lower RHs with the same conidial concentration. Both egg and adult mortality were higher at 95 ± 2% RH than at lower RHs, irrespective of conidial concentrations. It showed that an increasing RH value increases the efficacy of fungal entomopathogen. The best result was obtained when RH was higher, and the concentration was at the threshold level.

Finally, it can be concluded that the efficacy of the entomopathogen *B. bassiana* increases with raising of humidity. *Beauveria bassiana* is a promising alternative for the successful management of *T. macfarlanei* in IPM programmes. So, this study will contribute to developing an appropriate pest management strategy for spider mites using an entomopathogenic fungus considering humidity conditions. However, the field application of *B. bassiana* needs to be accurately evaluated for its effectiveness.

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Conflict of interest

The authors have no conflict of interest to declare.

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